Analytica Chimica Acta, 274 (1993) 25–35
Elsevier Science Publishers B V , Amsterdam

Crayfish walking leg neuronal biosensor for the detection of pyrazinamide and selected local anesthetics

Donal Leech and Garry A Rechnitz

Hawaii Biosensor Laboratory, Department of Chemistry, University of Hawaii at Manoa, 2545 The Mall, Honolulu, HI 96822 (USA)

(Received 6th October 1992)

Abstract

Neuronal biosensors based on the use of crayfish *Procambarus clarku* walking legs are reported for the first time and possible analytical applications are explored The neuronal biosensor is shown to respond selectively to the antitubercular drug and potent food marker, pyrazinamide The sensor is characterized with respect to selectivity, dose-response relationship, reproducibility and operating lifetime The detection of local anesthetics is accomplished by monitoring the decrease in pyrazinamide-induced nerve firing in the crayfish walking leg This decrease is a result of the local anesthetic block of the axonal sodium channels in the nerves Applicability of this novel procedure to analytical measurements is demonstrated by the construction of dose-response curves for several local anesthetics Problems associated with the current sensor configuration are presented and future research directions that may improve the neuronal-based biosensor are discussed

Keywords Biosensors, Crayfish, Pyrazinamide

In recent years researchers have recognised the analytical applicability of the unique binding properties of neuronal receptors Recent advances using isolated neuronal receptors, such as the nicotinic acetylcholine receptor (nAchR), include non-isotopic binding assays and the development of receptor-based biosensors Biosensors have been designed using the nAchR that detect ligand binding as a change in capacitance [1], impedance [2] or fluorescence [3,4] The difficulties associated with the isolation, purification, immobilization and stabilization of these receptors, however, has led to the investigation of intact receptors for biosensing applications A neuronal biosensor has been reported which utilizes the visceral ganglia of the pond snail for the detection of the neurotransmitter serotonin [5]

Correspondence to G A Rechnitz, Hawaii Biosensor Laboratory, Department of Chemistry, University of Hawaii at Manoa, 2545 The Mall, Honolulu, HI 96822 (USA)

Our approach has been to use intact chemoreceptor structures from the decapod crustacea for the detection of receptor stimulants This approach allows the sensing cells to remain in their, presumably optimized, native environment The chemoreceptors function by converting binding energy into electrical impulses (action potential spikes) that can be recorded by conventional electrophysiological microelectrode techniques Our earlier papers reported the application of antennules from the blue crab *Callinectus sapidus* to neuronal biosensing [6–8] All of our studies with the antennular chemoreceptor-based biosensors have recently been reviewed [9,10] These studies demonstrated the extreme sensitivity, high selectivity and broad response range that could be achieved using this approach Recently we reported the use of antennules from the freshwater crayfish *Procambarus clarku* for the selective detection of the antitubercular drug pyrazinamide [11] The freshwater crayfish offers better thermal

and time stability than the salt water crabs used in previous studies thus leading to longer viable sensor lifetimes and obviating the need for rigid control of the temperature

Electophysiological investigations of the pyridine receptors located in the walking leg of the crayfish *Austropotamobius torrentum* have shown pyrazinamide to be the most effective stimulant of action potential firing among the 79 pyridine analogs tested [12] In this communication we report on the first use of the the crayfish walking leg for the development of a chemoreceptor-based biosensor for the detection of pyrazinamide This paper also reports on a novel use of the intact-chemoreceptor biosensor for the detection of local anesthetics Local anesthetics (LAs) can be detected based on their ability to block the axonal sodium channel and thus their ability to decrease the excitability of nerve upon stimulation with the potent stimulant pyrazinamide

Electrophysiological and behavioral studies have revealed the presence of chemoreceptors on the antennules and both first and second walking leg (pereiopod) of the crayfish [13] Crayfish pereiopods are comprised of a two part protopod, the coxa and basis The endopod (the walking leg lacks an exopod) rises from the protopod and consists of five articles ischium, merus, carpus, propodus and dactylus In the first and second pereiopod the propopodite and dactylopodite form a pincers Chemosensory sensilla (aesthetascs) are located on the crayfish antennules and the first and second pereiopods A schematic depicting the structure of the crayfish first pereiopod is shown in Fig 1 Morphological investigation by Altner et al [14] has shown that each sensillum of the crayfish *A torrentum* consists of eight dendrites, six of which are chemosensitive, lying within a central canal The pore of the canal is plugged with electron dense material suggested to be mucopolysaccharides through which all stimulus molecules must diffuse to reach the dendrites The physiology of chemoreception has been presented in detail previously [6–11] and will not be discussed further here

The structure and pK_a of the LAs investigated in this report are depicted in Fig 2 Extensive

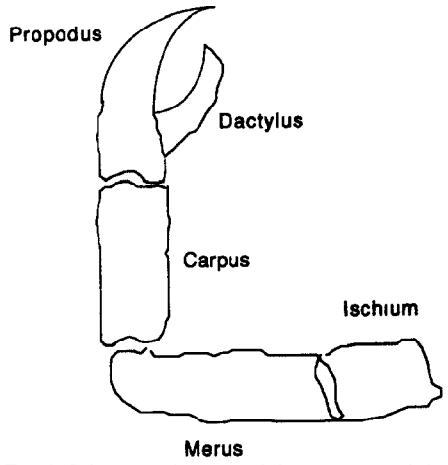

Fig 1 Schematic diagram of the structure of the crayfish first periopod

electrophysiological studies have shown that the mode of action of the LAs is to block the voltage-dependent sodium channel in nerve membranes resulting in a depression in the excitability of the nerve fibers [15] Several mechanisms have been proposed to describe the action of the LAs The simplest of these, the one-site model, proposes that both charged and uncharged LAs bind at a single site in the channel, leading to channel block A "modulated receptor hypothesis" has been proposed by several researchers [15–17] to account for the use-depen-

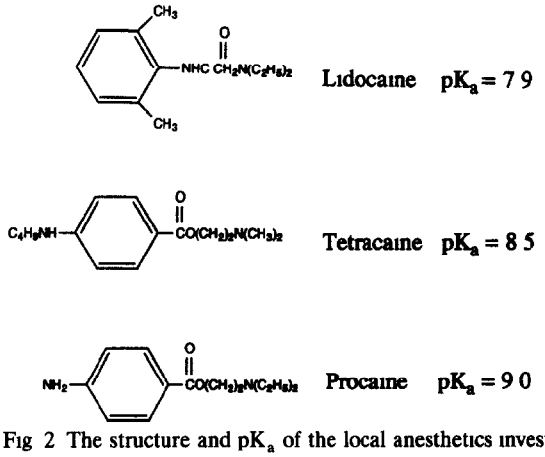

Fig 2 The structure and pK$_a$ of the local anesthetics investigated in this report

dent blocking properties and the wide variety in the potency and duration of action of the LAs This model postulates that each channel state (i e , open, resting or inactivated) can have different kinetics of interaction with the LAs The most rapid interaction of the LAs is for open channel block but their highest affinity is for inactivated forms of the channel Studies have shown the molecular size, lipophilicity and charge of the LAs to be important determinants of potency of sodium channel block [15–18]

Many methods, including polarography [19] and spectrophotometry [20] have been reported for the detection of LAs A recent paper reported the use of PVC-coated wire electrodes for the potentiometric determination of the cationic forms of the LAs [21] A rapid assay procedure for LA activity has been reported based on the competitive inhibition of [^{3}H]batrachotoxinin A 20α-benzoate binding to voltage-dependent sodium channels in a vesicular preparation from the guinea pig cerebral cortex [22] A non-isotopic assay procedure for the screening of LA activity and the quantitative detection of LAs is presented in this report This report introduces the novel application of the intact-chemoreceptor

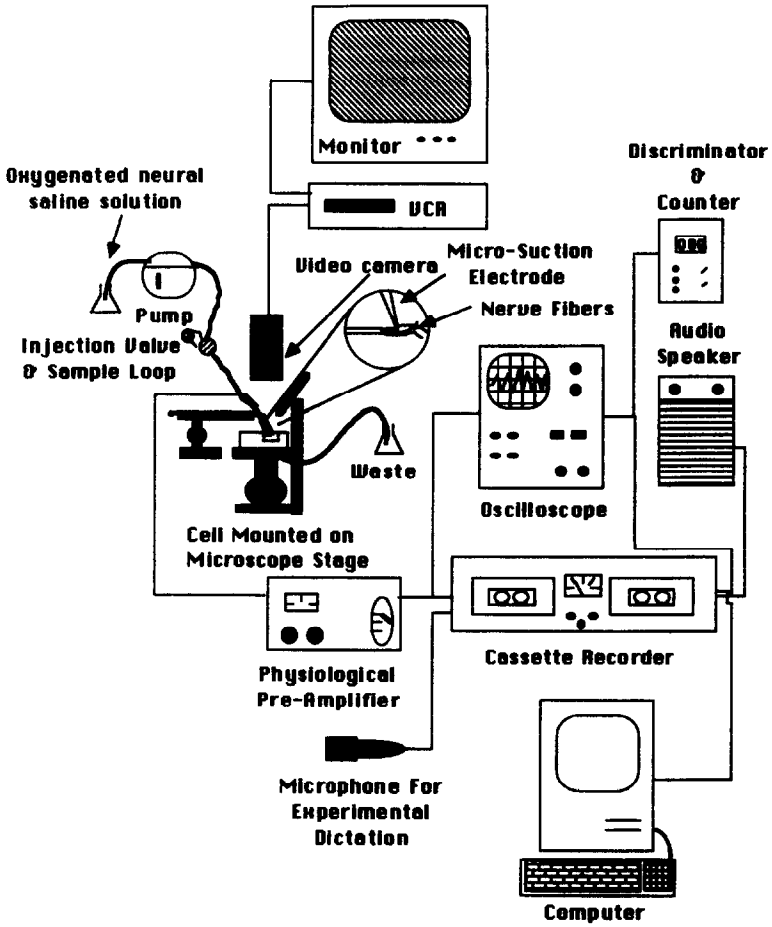

Fig 3 Schematic of the experimental set-up used to record the action potential firing of the nerves

biosensor for LA detection based on LA blockage of axonal sodium channels

EXPERIMENTAL

Reagents

The neural bathing solution and carrier was prepared according to a modified Van Harreveld formula and consisted of 205 mM NaCl, 5 4 mM KCl, 13 5 mM $CaCl_2$, 2 6 mM $MgCl_2$ and 10 mM Tris–malate buffer adjusted to pH 7 5 Stock solutions of the local anesthetics, procaine hydrochloride, lidocaine hydrochloride and tetracaine hydrochloride (Sigma), the pyridine analogues, pyrazinamide, 3-acetyl pyridine and nicotinamide (Sigma), and all other possible stimulants were prepared daily or as required in the neural bathing media Tracer studies were performed with a 2,6-dichlorophenolindophenol sodium salt (DCPIP, Sigma) dye solution in deionized water

Apparatus

A schematic diagram of the experimental setup is shown in Fig 3 The specially designed plexiglass cell was constructed to fit on the mechanical stage of a BioStar 1820 (Reichert-Jung) inverted biological microscope The microscope was capable of a maximum magnification of 450 × A video unit attached to the microscope consisting of a Hitachi VK150 colour video camera connected to a Sony Trinitron color video monitor extended the maximum magnification capability to 1700 ×, thus allowing visualization of the sensory aesthetascs and nerve fibers The video images were stored on video tape using a JVC HR-D660U video cassette recorder for subsequent photography with a Chinon CM-7 35-mm camera

The cell consisted of a circular chamber of 2 cm diameter, surrounded by a water jacket for temperature control The bottom of the cell was lined with a Sylgard 184 (Dow Corning) elastomer for the pinning of the preparations The flow into and out of the cell was controlled by the same peristaltic pump (Rainin) The bathing solution was introduced and removed through two ports located approximately 2 mm from the top of the chamber (total depth of 12 mm) and 45° to each other The outlet tubing was of a larger diameter than the inlet in order to maintain a constant operating volume in the cell Test solutions were introduced via a four-way valve (Rheodyne) equipped with a 0 25-ml sample injection loop located 20 cm from the inlet port Reference and ground wires were inserted into the cell and connected to a Grass P-15 physiological preamplifier Glass micropipettes were pulled to approximately 25 μm taper (Narishige PP-83 glass microelectrode puller) and flame polished by rapidly passing through the flame An Ag/AgCl electrode was inserted into the micropipette and positioned in the holder (E W Wright) and then placed in the micromanipulator (Narishige) and connected to the preamplifier The amplifier output was monitored on a Sony Tektronix 314 storage oscilloscope and also fed into one channel of a Yamaha KX-W900U stereo cassette deck for storage on audio tape A microphone enabled an oral account of the experiment to be recorded on the other channel of the audio tape and speakers attached to the stereo cassette recorder allowed aural monitoring of action potential firing Data analysis was accomplished by feeding the stored data into a window discriminator and digital event counter (Newark Electronics) for frequency counting Stored data was also fed into a Macintosh SE computer via a MacInstruments data acquisition package (G W Instruments) allowing the computer to function as a storage oscilloscope

Procedures

The freshwater crayfish *P clarku* could be conveniently captured in the Manoa stream located on the university campus The first pereiopod of the crayfish was excised at the ischium-merus joint, placed on a microscope slide and submerged in a drop of neural solution The hard carapace and connective muscle were removed from the merus and carpus portions of the walking leg to reveal approximately 1 cm of nerve fibers The whole preparation consisting of the propopodite and dactylopodite pincers and the nerve bundle was pinned into the cell, mounted on the microscope mechanical stage and con-

nected to the flow lines Oxygenated neural saline solution was circulated through the cell at all times in an effort to increase the viable lifetime of the sensing system The Ag/AgCl pickup electrode was positioned with the micromanipulator to gently contact the nerve fibers and mild suction was applied to the back of the micropipette to fill the electrode with electrolytic fluid and establish good electrical contact with the nerve fibers Spontaneous action potential firing of the nerves was then monitored on the oscilloscope (and over the speakers) to ensure good electrical contact and to check the viability of the nerves The injection of the various analytes was carried out and the frequency of action potential firing monitored If no response was observed the electrode was repositioned and the process repeated

Frequency of action potential firing (spike frequency) was monitored by counting the number of events falling in a set time period, usually 8 s, with the window discriminator and event counter Background (spontaneous) firing frequencies were also counted and subtracted from the response frequency Dose-response curves for pyrazinamide were obtained by injection of serially diluted stock solutions always commencing with the least concentrated to minimize receptor saturation, adaptational effects and nerve damage by this toxic stimulant The dose-response curves for pyrazinamide were constructed by plotting the maximum firing frequency versus concentration Inhibition curves for the LAs were obtained by injection of mixed solutions consisting of a constant pyrazinamide concentration, to elicit action potential firing, and variable LA concentrations The inhibition curves were constructed by plotting the decrease in the maximum pyrazinamide-induced firing frequency, or the decrease in action potential amplitude, versus concentration

Tracer studies were performed by injection of 1 mM of DCPIP into the chamber using deionized water as the carrier Samples of 50 μl were taken at intervals after the injection of DCPIP from the centre of the chamber and at the side of the chamber where the walking leg pincers were normally pinned The samples were diluted to 0 5 ml with deionized water and their absorbance measured at 600 nm on a Milton Roy Spectronics 1201 spectrophotometer The concentration was expressed as a percentage of the maximum concentration attained in the center of the chamber and related to the injected concentration by linear extrapolation Standard solutions of DCPIP were also prepared and their absorbance measured to ensure that the Beer–Lambert law was obeyed over the concentration range used

RESULTS AND DISCUSSION

Tracer studies

Tracer studies of the bathing cell using the dye DCPIP were performed in order to investigate the mixing process in the chamber and the relationship between the injected concentration and the maximum concentration reached in the chamber Tracer studies are also helpful in determining the washout time of the stimulant At the λ_{max} for DCPIP (600 nm) an ϵ_{600} of 16000 M^{-1} cm^{-1} was calculated from the linear absorbance–concentration curve for DCPIP concentrations up to 0 1 mM ($n = 6$, $r^2 = 0\,9999$) Sampling of the tracer solution was from a point in the center of the chamber where the nerve fibers were pinned and electrode contact with the nerve fibers was normally achieved and also from a position at the side of the chamber 90° from the inlet where the crayfish pincers that contain the chemosensory aesthetascs were normally pinned

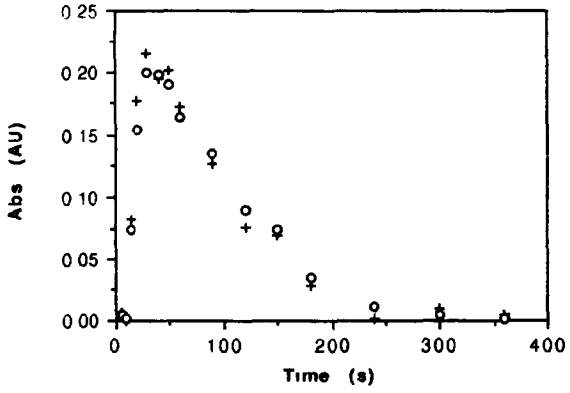

Fig 4 Time course of injected DCPIP in the cell at sampling positions in the center of the cell (+) and 90° from the inlet (○)

The time course of the injected dye solution at both sampling positions in the chamber is shown in Fig 4 The zero time indicates the time at which the sample injection loop was opened, thus explaining the 14-s time lag between injection and increase in response This figure shows that little variation is seen in the time profile of the dye solution between the two different sampling positions, demonstrating the good mixing achieved in the chamber The maximum in the response profile is attained approximately 35 s after injection of the tracer dye The actual maximum concentration achieved in the cell was calculated, by linear approximation, to correspond to 13% of the injected dye concentration All subsequent concentration values for pyrazinamide and the LAs refer to the injected concentration unless otherwise specified From Fig 4 the washout time required to remove all traces of the dye from the chamber was determined to be 4 min

Pyrazinamide detection

Action potential firing can be induced in response to a depolarizing stimulus This stimulus may be electrical (a depolarizing voltage or current pulse) or chemical (injection of a receptor agonist) in this study we utilize the potent stimu-

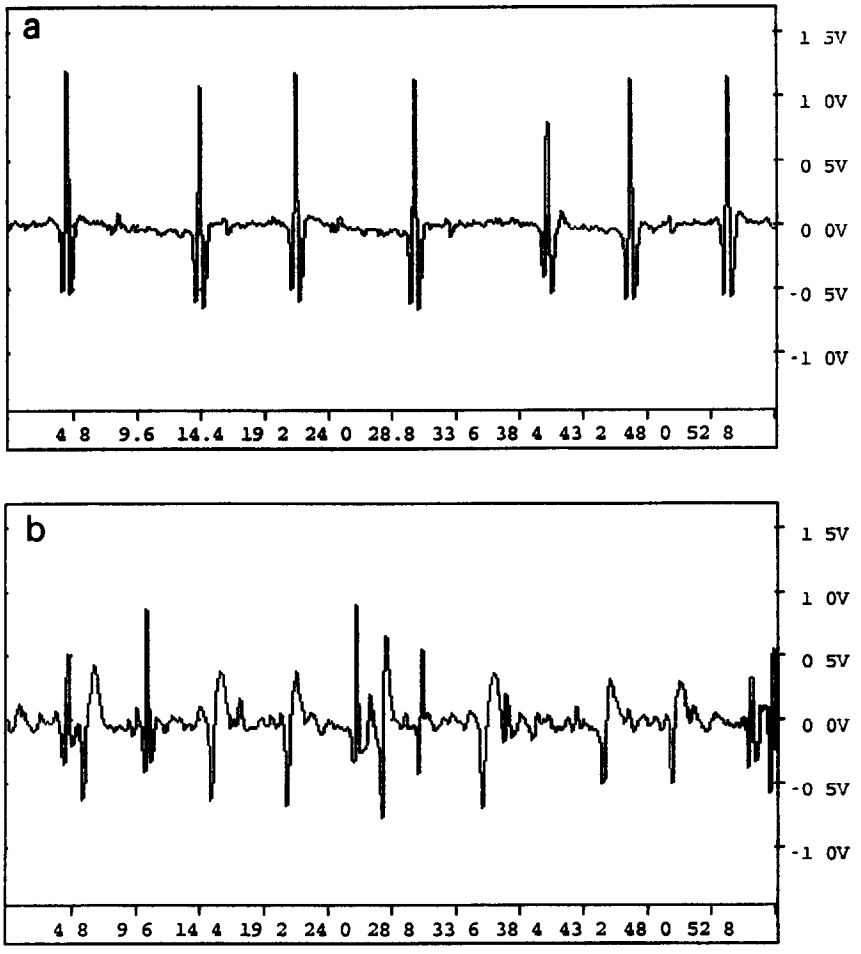

Fig 5 Single-unit (a) and multi-unit (b) response data recorded at different crayfish walking leg nerve fibers in response to the injection of 5×10^{-4} M pyrazinamide The data are shown as viewed with the MacInstruments data acquisition software after amplification and digitization

lant pyrazinamide to elicit action potential firing in the nerves for the detection of nerve block by the LAs The response of the crayfish antennular receptrode to this stimulant has been well characterized by our group [11] The application of the chemoreceptors located in the first pereiopod of the crayfish to the detection of pyrazinamide is investigated in the following section Properties of this pyridine chemoreceptor agonist which are favorable to its use as a stimulus for the detection of the LAs are also investigated

As previously reported [6–11] there are two types of response patterns that can be obtained with the extracellular recording techniques used in this study, the so-called multi-unit and single-unit response patterns These two response patterns are illustrated in Fig 5, which depicts the response (after amplification and digitization) obtained at the crayfish walking leg upon injection of 5×10^{-4} M pyrazinamide Multi-unit data consists of action potentials firing at various amplitudes in response to the stimulant This is because the pickup microelectrode is normally in contact with several nerve fibers and the action potential amplitude detected is dependent on the seal between the micropipette and the nerve fiber If only one nerve fires in response to injection of stimulant then a single-unit response pattern is observed, as shown in Fig 5a The analysis of single-unit data is simple, relying on the window discriminator to eliminate noise and the event counter to count the frequency of firing in response to stimulation application Multi-unit responses are more difficult to analyze, requiring careful consideration of the window for discrimination of unwanted signals

As discussed previously, an increase in the action potential firing frequency results from an injection of a chemoreceptor agonist A typical lag time between injection of pyrazinamide and the onset of increase in action potential firing of 45–60 s was seen, with the firing frequency reaching a maximum approximately 15–30 s later The lag time between maximum cell concentration (determined to be approximately 35 s after injection, from tracer studies above) and maximum firing frequency attainment may be attributed to the diffusion of the stimulant through the aesthetasc pore to reach the chemoreceptive dendrites, this lag has been reported previously for the detection of pyridine analogues at the crayfish *A torrentum* walking leg [12] The maximum firing frequency has been shown to be related to the concentration of applied agonist [6], allowing a dose-response curve of maximum firing frequency versus agonist concentration to be constructed for pyrazinamide detection at the crayfish walking leg A typical dose-response curve is shown in Fig 6 This curve indicates that an increase in firing frequency over background firing is observed for injections of pyrazinamide concentrations above 1×10^{-5} M The dose-response curves show linear responses for concentrations ranging from 1×10^{-4} M up to 5×10^{-4} M A response plateau is reached at concentrations above 5×10^{-4} M, possibly indicating receptor saturation The dose-response curves obtained at the crayfish walking leg are similar to those reportedly obtained at the crayfish antennule [11] In that study a different cell configuration was used which involved a flow injection type system with minimal dilution of injected agonist If the walking leg dose-response curves are corrected for stimulant dilution in the cell system utilized in this study (vide supra) an ED_{50} value (concentration of half-maximal response) of approximately 2.8×10^{-5} M is obtained This ED_{50} value is close to that obtained at the antennular receptrode [11] indicating the homogeneity of this

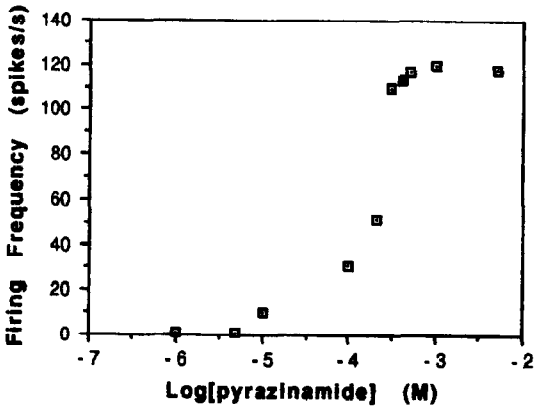

Fig 6 Dose-response curve obtained at the crayfish walking leg for pyrazinamide

chemoreceptor response from structure to structure

The crayfish pyridine-sensitive units also respond to other pyridine analogues and a detailed study of the structure–activity relationship of this receptor in the walking legs of the *A torrentum* crayfish has been reported [12] The order of potency for the three pyridine based stimulants, pyrazinamide > 3-acetylpyridine > nicotinamide, tested in this study at the *P clarku* walking leg was similar to that at the *A torrentum* walking leg The selectivity of the pyridine-sensitive units in the walking leg of *P clarku* for pyridine analogues was investigated by injecting solutions of other possible stimulants This included injections of neurotransmitters, excitatory amino acids, hormones, essential amino acids and other possible food markers No response at the pyridine-sensitive units for any of these solutions was observed indicating the high selectivity that can be achieved with the chemoreceptor-based biosensor

From the dose-response behaviour of pyrazinamide at the walking leg chemoreceptor, a pyrazinamide concentration of 5×10^{-4} M was selected to elicit axonal action potential firing in order to detect channel block by the local anesthetics This concentration stimulates the maximum firing frequency response while minimizing the nerve poisoning that can result from injections of this toxic drug The reproducibility of repeated injections of this concentration of pyrazinamide was investigated to ensure ample time was allowed for recovery of the nerves between injections and to assess the working lifetime of the sensor for the detection of the LAs The time profile resulting from repeated injections of 5×10^{-4} M pyrazinamide is shown in Fig 7 In this study a 10-min rinse time between injections was utilized as shorter rinse times resulted in a rapid desensitization leading to a decrease in firing frequency maxima with each successive injection Figure 7 demonstrates the reproducibility of the biosensor for repeated pyrazinamide injections Slight variations in the firing frequency maximum occur over the first 9 injections, yielding a mean firing frequency maximum value of 71 spikes s^{-1} (relative standard deviation of 10%) The decrease in the maximum firing frequency with subsequent

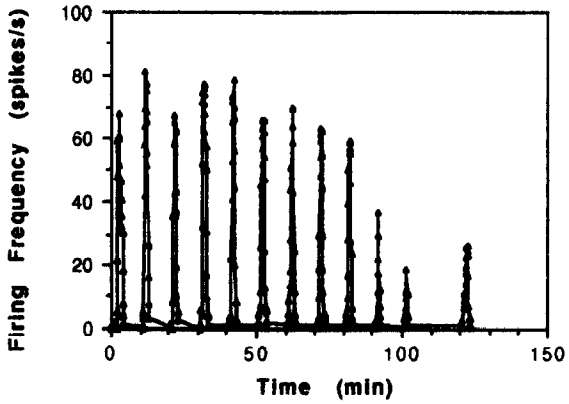

Fig 7 Time profile of the neuronal firing frequency in response to repeated injections of 5×10^{-4} M pyrazinamide

injections may be attributed to nerve damage caused by the repeated injections of the toxic pyrazinamide and/or by nerve damage resulting from the stress associated with the suction microelectrodes Adaptational events also contribute to the observed decrease as seen in Fig 7 from the resulting slight recovery in the firing frequency maximum upon increasing the rinse time to 20 min The operating lifetime of the biosensor can thus be assessed to be limited to approximately 2 h An improvement in the viable lifetime of the dissected nerves by incubation in oxygenated saline solution, to approximately two days has been achieved [23] However, extension of the operating lifetime of the system using the current invasive microelectrode detection procedures has not been achieved

Local anesthetic detection

The detection of the LAs can be achieved by monitoring the decrease in elicited excitability of the nerves upon injection of increasing LA concentrations Monitoring the decrease in spontaneous background firing of the nerves upon injection of the LAs proved unfeasible because of the low rate of spontaneous firing and the variability in firing frequency within and between nerve populations Response curves were generated by monitoring the decrease in neuronal activity, as described below, upon injection of mixed solutions of the stimulant, 5×10^{-4} M pyrazinamide,

and the LA Rinse times of 10 min were normally allowed for the recovery from block by the nerves except for concentrations of LA higher than 1×10^{-4} M, when longer rinse times were required

Two data analysis procedures were adopted for the construction of inhibition curves for the LAs at the crayfish walking leg, depending on the type of response elicited by injection of pyrazinamide For the single-unit data response patterns, inhibition curves could be constructed from the decrease in the detected compound action potential amplitude with increasing LA concentration This decrease is a result of the increasing block of axonal sodium channels with increasing LA concentration Inhibition curves for the local anesthetics lidocaine and tetracaine constructed in this manner are shown in Fig 8 For the multi-unit response patterns it was difficult to resolve the responding nerve action potential spikes from each other and the background, thus a different data analysis approach was devised Using the window discriminator, a cut-off action potential amplitude was selected and all of the action potential spikes falling within the window were counted Upon injection of increasing concentrations of the LAs an increasing proportion of the action potential spikes fail to reach the minimum amplitude required to be counted Thus inhibition curves, similar to those obtained from the single-unit response data, can be constructed from the decrease in the firing frequency maximum with injection of increasing LA concentrations Inhibition curves for procaine, lidocaine and tetracaine analyzed in this manner are presented in Fig 9 The response curves are affected by the choice of the discrimination window and this is reflected in the variability of the results from preparation to preparation Other factors, such as differences in nerve fiber diameters [24] and the use-dependent blocking properties of the LAs [15–18] may also contribute to the variability in inhibition curves from nerve to nerve

From Figs 8 and 9 IC_{50} values of approximately 2×10^{-6} M for both lidocaine and tetracaine and 5×10^{-5} M for procaine can be determined The relative blocking potency of the LAs has been shown to be related to the pH of the bathing solution and also to the relative hydrophobicity of the LAs [15–18] Because both lidocaine and tetracaine have lower pK_a values than procaine, a higher proportion of these drugs will exist in the more potent neutral form thus explaining their relative potencies The similar potency of action between tetracaine and lidocaine even with the differences in pK_a values can be attributed to the more hydrophobic nature of tetracaine thus allowing this LA to interact with the channel to the same extent as lidocaine In fact other workers have reported on the higher potency of tetracaine with respect to lidocaine,

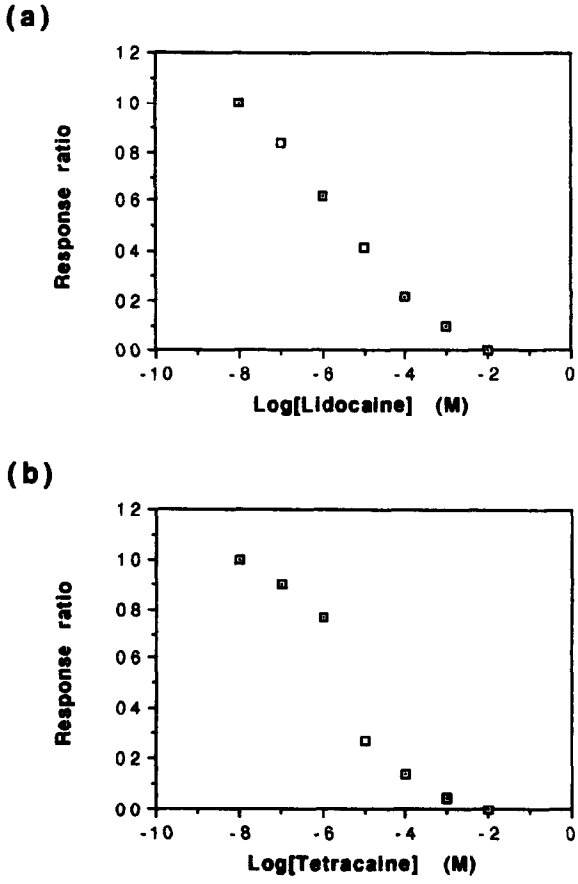

Fig 8 Inhibition curves constructed from the decrease in action potential amplitude upon injection of increasing concentrations of the local anesthetics lidocaine (a) and tetracaine (b) The response ratio is the ratio between action potential amplitude obtained upon injection of pyrazinamide/local anesthetic mixed solutions and the action potential amplitude obtained for injections of pyrazinamide alone See text for further details

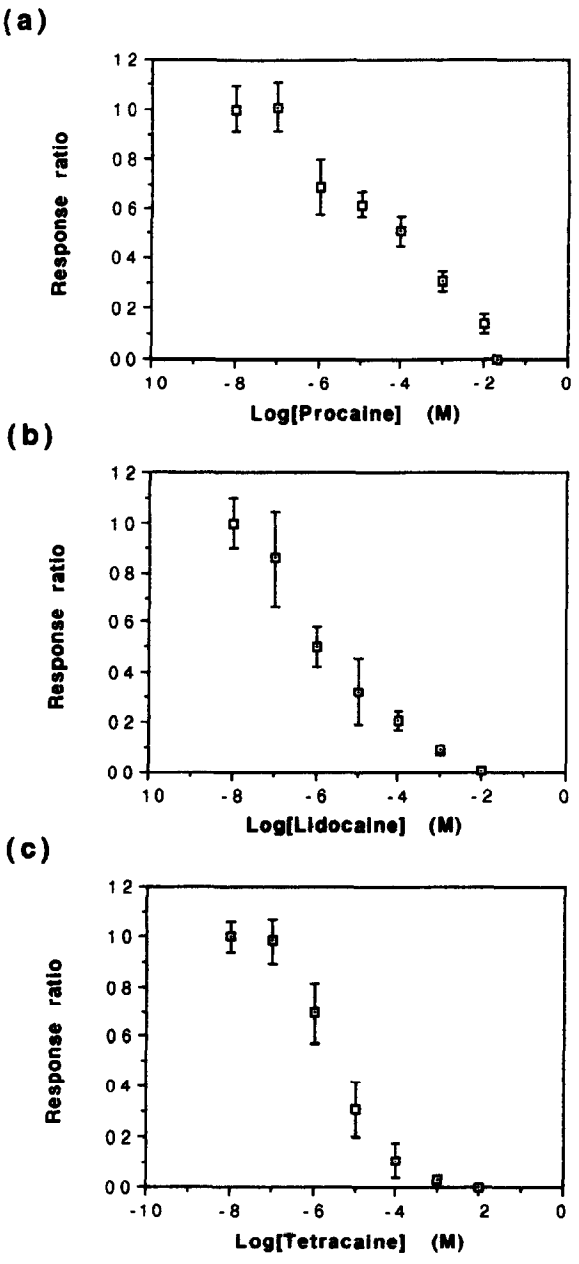

Fig 9 Inhibition curves constructed from the decrease in the firing frequency in response to stimulant injection upon injection of increasing concentrations of the local anesthetics procaine (a), lidocaine (b) and tetracaine (c) The response ratio is the ratio between the firing frequency obtained upon injection of 5×10^{-4} M pyrazinamide/local anesthetic (variable concentration) mixed solutions and that obtained upon injection of 5×10^{-4} M pyrazinamide alone See text for further details

with the relative potency of the three local anesthetics investigated in these studies, based on voltage-clamped frog nerve preparations [18] and competitive binding of the LAs with radio-labelled batrachotoxin at sodium channels in a vesicular preparation from guinea pig cerebral cortex [22], determined to be tetracaine > lidocaine > procaine The discrepancies between the previously reported potencies and those reported here may be attributed to the above mentioned variables and also to the different detection methods and preparations utilized in these studies Future research into the application of intact receptors for the detection and activity assay of channel blockage in order to improve the procedure is discussed below

Conclusion

This report demonstrates the utility of the chemoreceptors based in the walking leg of the crayfish for the detection of the antitubercular drug pyrazinamide This drug has been shown to elicit a potent reponse at the walking legs of the crayfish The properties of the response for pyrazinamide have been utilized for the development of a novel detection procedure at the walking legs This procedure demonstrates the utility of the chemoreceptor-based sensing system for the novel application of the detection of local anesthetic induced channel blockage As the nerve blocking properties of the LAs is related to their pharmacological activity this procedure has an added application in the possible screening of new local anesthetics Although there are several problems with the current procedure, such as limited lifetimes, variability of results and the tedious experimental procedures, future directions in this research could lead to a viable sensor for the LAs and a simple non-isotopic screening assay for the channel blocking activity of drugs Future work in this laboratory will focus on the use of electrical stimulation of the nerve fibers for detection of nerve block and on further possible procedures for the extension of the operating lifetimes of the chemoreceptor-based biosensors Promising possibilities in this direction include the use of alternative detection methods to the

invasive microelectrode techniques, such as voltage-sensitive dyes and the detection of the biomagnetic field surrounding the nerve fibers upon stimulation of action potential firing

The assistance of D Wijesuriya is acknowledged Financial support from the National Science Foundation is gratefully acknowledged

REFERENCES

1 M E Eldefrawi, S M Sherby, A G Andreou, N A Mansour, Z Annau, N A Blum and J J Valdes, Anal Lett, 21 (1988) 1665
2 R F Taylor, I G Marenchic and E J Cook, Anal Chim Acta, 213 (1988) 131
3 K R Rogers, J J Valdes and M E Eldefrawi, Anal Biochem, 182 (1989) 353
4 K.R Rogers and M E Eldefrawi, Biosensors Bioelectronics, 6 (1991) 507
5 R S Skeen, W S Kisaalita, B J Van Wie, S J Fung and C D Barnes, Biosensors Bioelectronics 5 (1990) 491
6 S L Belli and G A Rechnitz, Anal Lett, 19 (1986) 403
7 S L Belli and G A Rechnitz, Fresenius' Z Anal Chem, 331 (1988) 439
8 R M Buch and G A Rechnitz, Anal Lett, 22 (1989) 2685
9 R M Buch and G A Rechnitz, Anal Chem, 61 (1989) 533A
10 D Leech and G A Rechnitz, Electroanalysis, in press
11 D Wijesuriya and G A Rechnitz, Anal Chim Acta, 256 (1992) 39
12 H Hatt and I Schmiedel-Jakob, J Comp Physiol A, 154 (1984) 855
13 M S Laverack, in M A Ali (Ed), Nervous System in Invertebrates, Plenum Press, New York, 1987, pp 323–352
14 I Altner, H Hatt and H Altner, Cell Tissue Res, 228 (1983) 357
15 B Hille (Ed), Ionic Channels of Excitable Membranes, Sinauer, Sunderland, MA, 1984
16 B Hille, J Gen Physiol, 69 (1977) 497
17 L M Hondeghem and B G Katzung, Ann Rev Pharmacol Toxicol, 24 (1984) 387
18 K R Courtney, J Pharmacol Exp Therapeutics, 213 (1980) 114
19 V V Ogutsov and L P Yavors'ka, Farm Zh, (1986) 62
20 M E El-Kommos and K.M Emara, Analyst, 112 (1987) 1253
21 H Satake, T Miyata and S Kaneshina, Bull Chem Soc Jpn, 64 (1991) 3034
22 E T McNeal, G A Lewandowski, J W Daly and C R Creveling, J Med Chem, 28 (1985) 381
23 D Wijesuriya and G A Rechnitz, Anal Chim Acta, 264 (1992) 189
24 A Staiman and P Seeman, Can J Physiol Pharmacol, 52 (1974) 535